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Examiner:

For: T CELL RECEPTOR VARIANTS
EXPRESSED IN MESENCHYMAL
CELLS AND USES THEREOF

Attorney Docket No.: 85189-4900

SUBMISSION OF CERTIFIED PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
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Sir:

Applicants have claimed priority of Israeli application no. IL141539 filed February 20, 2001, under 35 U.S.C. § 119. In support of this claim, a certified copy of said application is submitted herewith.

No fee or certification is believed to be due for this submission. Should any fees be required, however, please charge such fees to Winston & Strawn LLP Deposit Account No. 50-1814.

Respectfully submitted,


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בקשה לפטנט
Application For Patent

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בעל אמצעה מכח העברת Assignment
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מולקולות DNA ותאים שנבררו טרנספקציה על ידן (בעברית)
(Hebrew)

DNA Molecules and Cells Transfected Therewith (באנגלית)
(English)

ה-Requested by me to be granted to me in respect thereof. בזאת כי ניתן לי עליה פטנט

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DNA MOLECULES AND CELLS TRANSFECTED THEREWITH

מולקולות DNA ותאים שעברו טרנספקציה על ידן

**Yeda Research and Development Co. Ltd. and
Bar-Ilan Research and Development Company Ltd.**

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ידע חברה למחקר ופיתוח בע"מ
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DNA MOLECULES AND CELLS TRANSFECTED THEREWITH

FIELD OF THE INVENTION

The present invention relates to cDNA molecules encoded by T cell receptor (TCR) genes and to antisense DNA molecules of said cDNAs and their use in the modulation of mesenchymal cell growth and cell functions. It further relates to the novel proteins, or peptides encoded by these transcripts, and uses thereof.

DESCRIPTION OF THE RELATED ART

10 MHC-restricted T cells express heterodimeric surface protein receptors ($\alpha\beta$ TCR) co-localized with up to five additional non-variant membrane receptors (Strominger, 1989; Abbas et al., 1994; Jameson et al., 1995). This TCR complex specifically binds processed peptide antigens associated with MHC molecules. The interactions of TCR with MHC bound peptides on various target cells may have consequences both in terms of T cell 15 proliferation and in activation of effector mechanisms leading to target cell killing, graft rejection, and other biological effects.

20 MHC class I gene products are widely expressed by various cell types while MHC class II molecules are expressed constitutively or are inducible in fewer, yet rather diverse cell types, such as dendritic cells, B lymphocytes, macrophages and vascular endothelial cells. By contrast, the T cell receptor complex is thought to be expressed solely by T cells, which further possess complicated signaling cascades as well as specific enzymes engaged 25 in TCR gene rearrangement. Thus, recognition of MHC presented peptides seems to be a highly specific T cell function.

Functional TCR α and β chain genes, which are capable of being expressed as 25 polypeptides, are normally present only in cells of the T lymphocyte lineage. These functional TCR genes are formed by somatic rearrangement of germline gene segments. Each TCR locus consists of variable (V), joining (J), and constant (C) region genes, and the β chain locus contains diversity (D) gene segments. In mice there are 20 to 30 V β gene segments that are located 5' of the two clusters of C and J segments. There is a single C α 30 gene associated with a large 5' cluster of up to 50 different J segments and about 75 V α segments. There is a large region of intervening DNA between V α and J α exons, which includes the entire TCR δ chain locus. During maturation of T cells in the thymus, the TCR segments are rearranged in a defined order, resulting in the formation of functional

TCR α and β genes in which V, D, J and C segments are in close proximity to each other.

The β chain locus rearranges prior to the α locus. The primary transcripts contain noncoding intronic sequences between the VDJ and C genes, which are later spliced out.

The functional T cell receptor is comprised of 2 polypeptides: the α chain is a 40 to 60 kD acidic glycoprotein, and the β chain is a 40 to 50 kD uncharged or basic glycoprotein. The 5 V and C regions of α and β chains form intrachain disulfide bond loops, which might contribute to the formation of a tertiary structure and are presented on the cell membrane.

The C region contains the transmembrane domain and a short cytoplasmic tail thought to be too small to have intrinsic signal transducing properties.

10 T cells (Qian et al., 1993; Yoshikai et al., 1984) as well as B cells (Calman and Peterlin, 1986) express a series of incomplete transcripts of TCR α and β , that vary in size and structure. These transcripts may be out of frame or their sequence may contain many stop codons. In some cases mRNAs encoding the constant region flanked by an upstream spliced J segment were identified. In one case such a transcript of human TCR β , which 15 contains an in-frame codon for methionine has been reported (Fagioli et al., 1991). However, no evidence for the existence of a protein encoded by these transcripts in T cells has been documented. TCR transcripts have also been reported in cell lineages other than T or B lymphocytes. Thus, TCR α mRNA was identified in murine kidney (Madrenas et al., 1992; Madrenas et al., Transplantation Proceedings 23: 837, 1991; Madrenas et al., 1994).

20 A recent study identified in epithelial tumor cells a partial TCR γ chain mRNA, lacking the V region. This mRNA encodes a 7 kDa protein, TARP, which is translated from a alternate reading frame and is therefore not homologous to the TCR γ protein (Essand et al., 1999; Wolfgang et al., 2000). No evidence for TCR $\alpha\beta$ or TCR δ transcripts or proteins was found in this study. It is therefore generally accepted that TCR β transcripts are not found 25 outside of the lymphocyte lineage and that TCR protein expressed at the cell surface is a specific T cell trait.

30 Mesenchymal cells play a central role in embryogenesis by directing organogenesis. In the adult organism, tissue remodeling, such as that occurring in wound healing, are initiated by mesenchymal fibroblasts. The study of regulation hemopoiesis demonstrated that blood cell formation is locally regulated by stromal mesenchyme (Zipori, 1989; Zipori et al., 1989; Zipori, 1990; Weintraub et al., 1996). Indeed, bone marrow-derived primary stroma as well as a variety of mesenchymal cells lines derived from primary bone marrow cultures exhibit an in vitro capacity to support hemopoiesis

and, upon transplantation, promote the formation of bone and hemopoietically active tissue in vivo at the site of transplantation. The molecules that mediate the instructive stromal activities have been shown to be a variety of cytokines and adhesion molecules. However, the molecules identified thus far cannot account for the wide spectrum of 5 stromal cell functions and certainly do not explain stroma organization, stem cell renewal and other vital stromal functions.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on 10 the information available to the applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

15 The present invention relates to new mRNAs transcripts and encoded proteins, which are short versions of α and β chains of the T cell receptor (TCR) as detailed herein below, and to uses of these molecules.

20 The present invention relates, in one aspect, to a cDNA molecule encoded by a T cell receptor (TCR) gene, said cDNA molecule lacking V region sequences and comprising a constant (C) domain and a joining (J) region sequences, and a 5' intronic J sequence upstream said J region sequence including an in-frame methionine codon.

The novel polynucleotide sequences disclosed herein and the corresponding proteins, polypeptides or peptides encoded by these polynucleotide sequences may be derived from any mammalian species including human genetic material.

25 In one embodiment of the invention, the cDNA molecule is encoded by a mouse TCR β gene. The joining (J) gene sequence may be selected from, but is not limited to, J β 2.1 and J β 2.6.

30 According to this embodiment of the invention, the joining (J) gene sequence may be J β 2.1 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the sequence M E N V S N P G S C I E E G E E R G R I L G S P F L as depicted in Fig. 4. In an alternative, the joining (J) gene sequence is J β 2.6 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the

sequence M G E Y L A E P R G F V C G V E P L C as depicted in Fig. 4. The cDNA molecule in a preferred embodiment has the nucleotide sequence depicted in Fig.1.

In another embodiment of the invention, the cDNA molecule is encoded by a TCR α gene. In this case, the joining (J) gene sequences are selected from, but not limited 5 to, J α TA31, J α TA46, J α New05, J α S58, J α New06, J α New08, J α LB2A, J α DK1, and J α TA39.

According to this embodiment of the invention, the cDNA molecule having a 5' intronic J sequence including an in-frame methionine codon is selected from the group consisting of:

10 (i) the intronic J α TA31 gene sequence coding for the peptide:

M A W H

(ii) the intronic J α TA46 gene sequence coding for the peptide:

M E A G W E V Q H W V S D M E C L T V

(iii) the intronic J α TA46 gene sequence coding for the peptide:

15 M E C L T V

(iv) the intronic J α New05 gene sequence coding for the peptide:

M T V

(v) the intronic J α S58 gene sequence coding for the peptide:

M C G S E E V F V V E S A

20 (vi) the intronic J α New06 gene sequence coding for the peptide:

M A C Y Q M Y F T G R K V D E P S E L G S G L

E L S Y F H T G G S S Q A V G L F I E N M I S T

S H G H F Q E M Q F S I W S F T V L Q I S A P G

S H L V P E T E R A E G P G V F V E H D I

25 (vii) the intronic J α New06 gene sequence coding for the peptide:

M Y F T G R K V D E P S E L G S G L E L S Y F H

T G G S S Q A V G L F I E N M I S T S H G H F Q E

M Q F S I W S F T V L Q I S A P G S H L V P E T E

R A E G P G V F V E H D I

30 (viii) the intronic J α New06 gene sequence coding for the peptide:

M I S T S H G H F Q E M Q F S I W S F T V L Q I S

A P G S H L V P E T E R A E G P G V F V E H D I

(ix) the intronic J α New06 gene sequence coding for the peptide:

MQ F S I W S F T V L Q I S A P G S H

L V P E T E R A E G P G V F V E H D I

(x) the intronic J α New08 gene sequence coding for the peptide:

M W W G L I L S A S V K F L Q R K E I L C

5 (xi) the intronic J α LB2A gene sequence coding for the peptide:

M V G A D L C K G G W H C V

(xii) the intronic J α DK1 gene sequence coding for the peptide:

M R E P V K N L Q G L V S

(xiii) the intronic J α TA39 gene sequence coding for the peptide:

10 M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(xiv) the intronic J α TA39 gene sequence coding for the peptide:

M E T G R E R S H F V K T S L.

According to an alternative and more preferred embodiment, the novel intronic sequences and their corresponding peptides may be derived from human genetic material. Any known sequences, such as the joining segment of human J β 2.3 gene, are explicitly excluded from the claimed novel sequences.

According to this embodiment of the invention, the cDNA molecule having a 5' intronic J sequence including an in-frame methionine codon is selected from the group consisting of:

1) the intronic J β 2.3 gene sequence coding for the peptide:

M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V

2) the intronic J α 2 gene sequence coding for the peptide

25 M

3) the intronic J α 3 gene sequence coding for the pep tide:

M L L W D P S G F Q Q I S I K K V I S K T L P T

4) the intronic J α 6 gene sequence coding for the peptide:

M L P N T M G Q L V E G G H M K Q V L S K A V L T V

30 5) the intronic J α 6 gene sequence coding for the peptide:

M G Q L V E G G H M K Q V L S K A V L T V

6) the intronic J α 6 gene sequence coding for the peptide:

M K Q V L S K A V L T V

7) the intronic J α 8 gene sequence coding for the peptide:

M S E C

8) the intronic J α 9 gene sequence coding for the peptide:

M A H F V A V Q I T V

5 9) the intronic J α 11 gene sequence coding for the peptide:

M G I C Y S

10) the intronic J α 13 gene sequence coding for the peptide:

M K R A G E G K S F C K G R H Y S V

11) the intronic J α 14 gene sequence coding for the peptide:

10 M L T T L I Y Y Q G N S V I F V R Q H S A

12) the intronic J α 24 gene sequence coding for the peptide:

M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V

13) the intronic J α 25 gene sequence coding for the peptide:

M

15 14) the intronic J α 31 gene sequence coding for the peptide:

M G F S K G R K C C G

15) the intronic J α 36 gene sequence coding for the peptide:

M K K I W L S R K V F L Y W A E T L

16) the intronic J α 40 gene sequence coding for the peptide:

20 M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

17) the intronic J α 40 gene sequence coding for the peptide:

M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

18) the intronic J α 40 gene sequence coding for the peptide:

M E S K A A S I N G N I M L V Y V E T H N T V

25 19) the intronic J α 40 gene sequence coding for the peptide:

M L V Y V E T H N T V

20) the intronic J α 41 gene sequence coding for the peptide:

M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E

G T W W L L Q G V F C L G R T H C

30 21) the intronic J α 41 gene sequence coding for the peptide:

M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G

R T H C

22) the intronic J α 44 gene sequence coding for the peptide:

M E S Q A T G F C Y E A S H S V

In another aspect, the invention relates to antisense DNA molecules of any of the cDNA molecules of the invention described above.

5 The invention further relates to expression vectors comprising the cDNA and antisense molecules of the invention, and to host cells, particularly mammalian cells, comprising said vectors. In one preferred embodiment the host cells are transfected mesenchymal human cells.

10 The cDNA of the invention can be used to transfet mesenchymal human cells for inducing mesenchymal cell growth. Thus the invention relates to compositions comprising said transfected mesenchymal human cells for use in disorders requiring induction of mesenchymal cell growth, such as wound healing.

15 The invention further relates to a method for inducing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a cDNA molecule according to the invention, in an amount effective to induce mesenchymal cell growth. This method is preferably applicable for enhanced wound healing.

20 The antisense DNA molecules of the invention can be used to transfet mesenchymal human cells for inhibiting or suppressing mesenchymal cell growth. Thus the invention relates to compositions comprising said transfected mesenchymal human cells for use in disorders requiring inhibition or suppression of mesenchymal cell growth, such as in carcinomas.

25 The invention further relates to a method for suppressing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising an antisense DNA molecule of the invention, in an amount effective to suppress mesenchymal cell growth, such as for suppression of carcinomas.

30 The invention further relates to a polypeptide encoded by a DNA molecule of the invention. In one embodiment, said polypeptide is a protein capable of being expressed on the cell surface or intracellularly, and is encoded by the nucleotide sequence depicted in Fig. 1, more preferably, the protein of the amino acid sequence depicted in Fig. 1.

The invention still further relates to a synthetic peptide deduced from an intronic J sequence of a TCR.

Examples of such peptides derived from non-human animals include but are not limited to:

(a) M E N V S N P G S C I E E G E E R G R I L G S P F L

(b) M G E Y L A E P R G F V C G V E P L C

5 (c) M A W H

(d) M E A G W E V Q H W V S D M E C L T V

(e) M E C L T V

(f) M T V

(g) M C G S E E V F V V V E S A

10 (h) M A C Y Q M Y F T G R K V D E P S E L G S G L

ELS Y F H T G G S S Q A V G L F I E N M I S T

S H G H F Q E M Q F S I W S F T V L Q I S A P G

S H L V P E T E R A E G P G V F V E H D I

(i) M Y F T G R K V D E P S E L G S G L E L S Y F H

T G G S S Q A V G L F I E N M I S T S H G H F Q E

M Q F S I W S F T V L Q I S A P G S H L V P E T E

R A E G P G V F V E H D I

(j) M I S T S H G H F Q E M Q F S I W S F T V L Q I S

A P G S H L V P E T E R A E G P G V F V E H D I

20 (k) M Q F S I W S F T V L Q I S A P G S H

L V P E T E R A E G P G V F V E H D I

(l) M W W G L I L S A S V K F L Q R K E I L C

(m) M V G A D L C K G G W H C V

(n) M R E P V K N L Q G L V S

25 (o) M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(p) M E T G R E R S H F V K T S L.

Examples of useful peptides according to the present invention derived from human sources include but are not limited to:

30

i) M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V

ii) M L L W D P S G F Q Q I S I K K V I S K T L P T

iii) M L P N T M G Q L V E G G H M K Q V L S K A V L T V

iv) M G Q L V E G G H M K Q V L S K A V L T V

v) M K Q V L S K A V L T V

vi) M S E C

vii) M A H F V A V Q I T V

viii) M G I C Y S

5 ix) M K R A G E G K S F C K G R H Y S V

x) M L T T L I Y Y Q G N S V I F V R Q H S A

xi) M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S
V

xii) M G F S K G R K C C G

10 xiii) M K K I W L S R K V F L Y W A E T L

xiv) M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

xv) M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

xvi) M E S K A A S I N G N I M L V Y V E T H N T V

xvii) M L V Y V E T H N T V

15 xviii) M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I
I G E G T W W L L Q G V F C L G R T H C

xix) M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C
L G R T H C

xx) M E S Q A T G F C Y E A S H S V

20

In still a further aspect, the invention relates to an antibody raised against a peptide as defined above, particularly against said peptide (b). These antibodies are useful as markers of mesenchymal cells, for example for diagnostic purposes and for prognosis of cancer.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the nucleotide sequence of the JintJ-C β 2 mRNA transcript of the stromal/mesenchymal cell line, MBA-13, and the deduced amino acid sequence encoded thereby. The cDNA products were obtained from reverse transcription (RT)-PCR analysis using TCR primers and sequenced.

Figs. 2A-2F show flow cytometric analysis of JintJ-C β 2 expression by mesenchymal cells. Mouse embryonic fetal fibroblast (2E) and different MBA-13 cell

strains (1-3; 2A-2C, respectively) were stained with preimmunized (black histogram) or immunized (green histogram) purified antibodies from rabbit serum. The rabbits were immunized to intronic J β 2.6 peptide (b) of the sequence M G E Y L A E P R G F V C G V E P L C. As a second antibody, we used Fab FITC conjugated donkey anti-rabbit IgG.

5 Staining with second antibody only gave an histogram shown in blue color. Cells stained with rabbit polyclonal antibodies to irrelevant peptide 1121 of the sequence RGGGGGRGGLHD, similarly produced and purified, served as negative control (brown histogram). Competition of antibody binding was performed by pre-incubation of the purified immune serum with the specific immunizing intronic-J β 2.6 peptide (b), for 30

10 min at room temperature (2D, red histogram). Competition with irrelevant peptide 1121 served as negative control (data not shown). The results of a single experiment, out of 3 performed, are shown.

Fig. 3 shows RT-PCR analysis of the novel TCRC β 2 cDNA including an in-frame 15 intronic J sequence designated J_{int}J-C β 2, obtained from MBA-13 mesenchymal cell line and fetal primary cell cultures. The cDNA was obtained from RNA extracted from mouse embryonic fetal fibroblast and different MBA-13 cell strains (1-3). RT-PCR was performed using the following sense pairs:

exonic J β 2.6: 5'-CTATAACAGTACTTCGGTC-3'; or
20 intronic J β 2.6: 5'-ATGGGAGAATACCTCGCTG-3'; or
5'-CCCTAAATGGGAGAATACC; and
antisense primer C β 3: 5'-CATCCTATCATCAGGGGGTTCTGTCTGCAA-3'.

Products of 465 bp and 524 bp were produced, respectively.

Fig. 4 depicts sequences of all possible versions of TCR α β containing an intronic 25 '5 end including an in-frame Met codon as collected from available data bases: the intronic J β sequences J β 2.1 and J β 2.6, and the intronic J α sequences J α TA31, J α TA46, J α New05, J α S58, J α New06, J α New08, J α LB2A, J α DK1 and J α TA39.

30 Fig. 5 shows determination of generation time of different clones of MBA-13 cell line. Eight individual clones were studied by PCR for expression of M-TCR (TCR β J^{int}-J_{2..6}C). Out of those, four were found to be negative (M-TCR clones E4, C6, G1, B7) and

four were found to be positive (M-TCR⁺ clones C4, D10, B10, B1). Cells were seeded for different concentrations (10^3 , 5×10^3 and 10^4 /ml) and cell growth was determined after 44 – 46 hours. The population's generation time was calculated.

5 **Figs. 6A-6C** show RT-PCR analysis of TCR expression in different cell lines and primary cell cultures. cDNA was obtained from RNA extracted from different cell types, as described in the Materials and Methods section hereinafter, and RT-PCR was performed using the following primer pairs: C β 1 and C β 2 primers for TCRC β 2 produced a 410 bp product (Fig. 6A); C α 1 and Tm or C α 1 and C α 2 for TCRC α produced a 356 bp
10 or 138 bp product, respectively (Figs. 6B and 6C).

15 **Figs. 7A-7D** show mRNA expression of TCRC β (7A-7B) , TCRC α (7C) and CD3 ϵ (7D) mRNA transcripts. Poly A+ mRNA, from mesenchymal (MBA-13, AC-6, NIH3T3, fetal cell culture), epithelial (1C8) and endothelial-adipocyte (14F1.1) cell lines, was Northern blotted as described in the Materials and Methods section hereinafter, and probed with the following probes: TCRC β , TCRC α and CD3 ϵ . For the TCRC β chain, thymus RNA exhibited 1.3 kb (full-length) and 1.0 kb (truncated) transcripts, while the mesenchymal MBA-13, AC-6 and fetal cells exhibited a 1.1 kb transcript (Figs. 7A and 7B). For the TCRC α chain, thymus RNA and non-T cell lines exhibited a 1.6 kb transcript (Fig. 7C). For the CD3 ϵ chain, thymus RNA exhibited a 1.5 kb transcript, while non-T cells showed a transcript whose size was slightly larger (Fig. 7D). Hybridization signals for TCRC β were quantitated by densitometric scanning, and the signal value of MBA-13 was 60 fold less than thymocytes.

25 **Figure 8.** shows flow cytometric analysis of CD3 ϵ , TCR $\alpha\beta$ and TCR $\gamma\delta$ antigens expression by MBA-13 cells. MBA-13 cells were stained with FITC-conjugated TCR $\alpha\beta$, CD3 ϵ and with PE-conjugated TCR $\gamma\delta$ (line). For intracellular staining, cells were fixed and stained with FITC-conjugated TCR $\alpha\beta$ using the Cytoperm kit. In all experiments, cells stained with isotype-matched FITC-conjugated rat anti-mouse IgG were also 30 prepared as negative controls (full line). The results of a single experiment are shown.

5 **Figure 9.** human TCR J2.3-C β . transcript was cloned from cDNA of cord blood mononuclear cells and amniotic fluid cells.. The cloned transcripts were sequenced and were found to be identical. The lines above the sequence indicate the boundaries of each segment. The predicted protein product is shown bellow the sequence. Bold font indicates an A to G transition that was found in both clones.

10 **Figure 10.** Expression of GFP-TCR J2.3-C β in 293T transfected cells. Western blot analysis. Each lane was loaded with lysate of 5×10^5 cells, GFP-TCR J2.3-C β . was detected with Anti-GFP monoclonal antibody JL-8 Clontech (Palo Alto, CA).

DETAILED DESCRIPTION OF THE INVENTION

15 The present invention relates to new mRNA transcripts and proteins encoded by these transcripts which are short versions of α and β TCR as detailed hereinabove in the Summary of the Invention section, and to uses of these molecules.

20 While studying the interactions of stromal cell lines with thymic T cells, we used reverse transcription polymerase chain reaction (RT-PCR) to amplify TCR gene fragments. Unexpectedly, the MBA-13 mesenchymal stromal cell line, derived from mouse bone marrow, was found to consistently express TCR β constant (C β) region, while cDNA from a negative control tissue, i.e. liver, and from several control cell lines such as pre-B cells, plasmacytoma and mastocytoma cells, did not produce PCR products using primers from the TCR gene.

25 Further studies with a variety of stromal cell lines, in accordance with the present invention, showed the existence of TCR gene derived mRNAs that encode short versions of the gene consisting of the constant (C) domain, which is identical to that of T cell receptor, a joining (J) region, which may be one of several alternatives, and a 5' domain consisting of a nucleotide sequence corresponding to an intronic J sequence (again one of 30 several alternatives) including an in-frame codon for methionine. This mRNA lacks V region sequences. One of such molecules, namely a new version of a TCR β 2.6, is shown herein to exist in mesenchymal cells and to encode a cell surface mesenchymal protein. Expression on the mRNA level has also been observed in the thymus.

According to the present invention, the uncloned stromal/mesenchymal mouse MBA-13 cell line was subdivided into substrains that either express or do not express the molecule of interest, i.e. the $J^{\text{int}}\text{-}J\beta 2.6\text{-}C$ protein and mRNA, on the mRNA and antigenic protein levels. We therefore single cell cloned MBA-13 cells and obtained 8 different 5 clonal populations by standard procedures. Out of these, 4 expressed the $J^{\text{int}}\text{-}J\beta 2.6\text{-}C$ protein (M-TCR $^+$ clones C4, D10, B10, B1) and 4 were negative (M-TCR $^-$ clones E4, C6, G1, B7). Fig. 5 shows that all the cells positive for $J^{\text{int}}\text{-}J\beta 2.6\text{-}C$ had a population 10 generation time (doubling time) of 15 hrs or less, which is considered very fast for mesenchymal cells. On the other hand, although the negative clones showed variable 15 results, all grew much slower and 2 clones had a very slow growth rate with doubling time between 36-38 hrs. It is therefore implied that the expression of the gene of interest correlates with fast growth rate and that lack of expression results in retarded growth. These results are supported by preliminary data indicating that antibodies to TCR β constant region interfere with the growth of mesenchymal cells.

Thus, the expression or lack of expression of the mesenchymal TCR seems to 15 control mesenchymal cell growth. The invention is therefore related to the use of the cDNA and antisense molecules of the invention derived from mesenchymal TCR mRNAs for expression in cells and tissues for the purpose of modulating stromal/mesenchymal cell growth and thereby their related tissue functions. For this purpose, the cDNA or antisense 20 molecule is inserted in appropriate vectors such as, but not limited to, the retroviral vectors DCA l and DCM m that are been used in clinical trials in gene therapy (Bordignon et al., 1995). Preferably, the vector containing the cDNA or the antisense molecule, under the 25 control of a suitable promoter such as that cDNA own promoter, will be used to infect or transfect suitable mammalian, preferably human, most preferably the patient's autologous mesenchymal cells. The thus obtained genetically-modified mesenchymal cells are then administered to a patient in need thereof by an appropriate route and are expressed in the desired site or tissue.

In one embodiment, for the treatment of wounds, local application of the cells 30 containing the cDNA molecule can be used to induce mesenchymal cell growth thus enhancing the wound healing process. In another embodiment, mesenchymal cells of the tumor can be transfected with the antisense cDNA and then be used for treatment of localized solid tumors, to achieve regression of the tumor mesenchyme and subsequent regression of the tumor.

The proteins encoded by the mRNAs of the invention are cell surface receptors of mesenchymal cells and may probably interact with ligands presented by neighboring hemopoietic or non-hemopoietic cells. Thus, in bound or soluble form, these proteins or the peptides derived therefrom, may have modulatory effects on cells that bear said 5 ligands.

The proteins and peptides of the invention may be used as immunogens for production of antibodies that may be used as markers of mesenchymal cells.

Thus the invention relates also to antibodies, particularly to polyclonal antibodies to the proteins and peptides of the invention as well as proteolytic fragments thereof such 10 as the Fab or F(ab')₂ fragments. These polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with said protein or peptide antigen.

The following are certain currently most preferred embodiments of human sequences according to the present invention. In these embodiments, the methionine 15 initiating the open reading frame is shown in bold italics, the amino acids that are translated from an intronic sequence upstream to J segments are shown in *italics* and the J segments are shown in bold, three dots denote the beginning of the C1 segments.

Homo sapiens beta gene segment

20 **J β 2.3 (bases 198551 to 198627)**
Met G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V S T D T Q Y F G P G
T R L T V L E D L K N V F P P E V A V F E P S E A E I S H T Q K A T L V C L A T
G F Y P D H V E L S W W V N G K E V H S G V S T D P Q P L K E Q P A L N D S R
25 *Y C L S S R L R V S A T F W Q N P R N H F R C Q V Q F Y G L S E N D E W T Q D*
R A K P V T Q I V S A E A W G R A D C G F T S E S Y Q Q G V L S A T I L Y E I L L
G K A T L Y A V L V S A L V L Met A Met V K R K D S R G Stop

Homo sapiens alpha gene segment

30 **J α 2 (bases 84269 to 84334)**

35 *L L F K Stop Stop V G P V S L C N G V T Y G Met N T G G T I D K L T F G K G T*
H V F I I S...

40 **J α 3 (83376. To 83437)**

L Q G I E A A Met Stop R E A H R P G E N L G S T L T G C F Q Stop S L H F L S S K
Met T I T T S Stop Stop Y E I Met A R Met Stop K V I N K Stop Stop LF Stop N I I I I I

IIEALLILRFTLS Stop RERRIASLGNKRCKQQRPKEPFR *Met L*
LWDPSGFQQISIKKVISKTLPTVGVQCFCQDNLWIRDQTQ
H P A...

5 **J_α6(79270 to 79331)**

QLQEKRHIKFPLLSVLAALSEAPCI Stop LKSSRARPSECLPQ
ASRVWCLYWGAGSRHGEELLPCFSADGKVVFSPGYTGAKE
LSSPQPLAPAPGLQHSGALRTAVGDELQLREYSGGFPR *Met*
10 LPNT *Met G* QLVEGGH *Met K* QVLSKA VLTVCIRRKLHTYIWKR
N Q P Y C S S ...

J_α8(76346 to 76405)

15 SIHGHHSCKKHV Stop L TN S Stop V W *Met V* K L P Stop VLSRTETL
Stop L Y Stop Stop L F Stop L E Y Stop Stop H F Y IT Q G I Q S R I F S W V L S D L
LSSSNGL R KIKVK Stop Stop D Stop Met PPTTLVHACRH R N T L S N
Stop L A C D L A I L A *Met A* Q Stop Q G P I L Y R V *Met S E C E H R L S E T C I*
W N W H P T S G Q S ...

20

J_α9(75756 to 75816)

25 Q Y N Stop S T R A Stop LLCEL Stop R N A G Stop R H F A H R T L A L R D S L K
ISSSPLFIFPIRKLRP R E V G Stop I V Stop G Q C E L G L G L E P G D P G P
G A I F C D C C L V N Stop T S D R Stop E V Stop V *Met L* I N R K N K Stop V L Q G
E Y K N V L L I T S T L V Stop A P Stop T C S P A V V Stop K W K E K E *Met A* H F
V A V Q I T V G N T G G F K T I F G A G T R L F V K A ...

30

B2

J_α11(72705 to 72765)

35 VNSGYSTLTFGKGTMLLVSP
E H C Y Stop S S D V W F Stop Q K N P N I A V I P L Stop K E Q G R G F F S E S S S
Stop D L S I L C Q S V L W I Q D T Y I F V S S A G P T C S A S D H L S L I C K *Met*
R I I F K L *Met A* Q L K P K Stop G S G I Y A D Y Stop S I W L I N E G F L S F S L C
R S W V E I P N T A N H F C *Met G* I C Y S V N S G Y S T L T F G K G T M L L V S
40 P ...

J_α13(71282 to 71342)

45 D Stop K I L E S Stop S Stop R K R Q K V W L S T S S S S D L A Stop L V N L G H S
I F I Y K *Met K* T F N I T S D F L F Stop F C G Y I I G V Y I Y F K D K L I Y V K V F
C K F L N A I H S E N I C L Stop N K K N Y V R F R I L L T Stop E F V G S Stop
Stop N S H L H V I C S P R H W Stop K A L S L L K Y S G S N A T Q *Met K* R A G
E G K S F C K G R H Y S V N S G G Y Q K V T F G I G T K L Q V I P ...

J_α14(70532 to 70583)

5 S Y S Met L L K K F Stop L I E E R K I I Y K D Met S N L L N S G K Met R L C T G V
D S **Stop** V K Met G V R A A I L W L V K Q D Y L V K L C K S P R K K **Stop** V S E
LS R E Y H L D C S Q A F H Y I Y C T T **Met** V P **Stop** K E A F S G L I P W L S L Y
S S I K K G E S S Q S S H E G D S C **Met** L T T L I Y Y Q G N S V I F V R Q H S A V I
Y S T F I F G S G T R L S V K P...

J_α24(60203 to 60265)

10 K T S S Y L N D R A T V V I S C H L S S A E D W V Stop P Stop V N A Stop A G G F
L S L Q H L K R T P R L H Stop P Q Q S G F L P L P P G R C S S W H T P S L V S
Stop K K R N **Stop** K R K G E K L I S H I **Met** Q L P H F V A R L F P H E Q F V F I Q
Q L S S L G K P F C R G V C H S V T T D S W G K L Q F G A G T Q V V V T P...

15 **J_α25(59046 to 59105)**
Q K D K A S P L S L G R G Q G C L S S Q
A Q A G G R K L Stop G V F A E P R N T V G I T **Met** V R I L S L V P E P D C P C C
P V S T V K W R Stop K Met S P V L D V G R S C R V L R P G V H R D L R S G D G
20 E E G Stop K R N E K Q N H K D N T E E G F I F G K E N H K A V Stop L T L E E
Met H S F G G S L L R R A L C R G K L S C **Stop** V F D A E I I T **Met** Q K D K A S P
L S L G R G Q G C L S S Q...

J_α31(51207 to 51263)

25 E L G W L C S W K I S L W V Stop E C T V P S N L C V Stop G Stop A H T Y D S K S
C Stop Q I R F S F G S F **Met** P R N A K E F Stop K L I S L A F L K E T L F A L C C R
A N F S S Y H K R P E T Q R K Q K K K R K K K K T Q G E S N C P L T T V L C V
W **Stop** G F T **Met** G F S K G R K C C G N N N A R L **Met** F G D G T Q L V V K P...

30 **J_α36(45351 to 45411)**
K L G A V S L T C N L S I L E G Stop G R R I T Stop G Q E F K T T L G N T V R P P
S L Q K I N K Stop N F F K N S Q A W Stop H A P V I L A T E E V E A G G S L V P R
35 R S R L Q Stop A K N T P L H S S L D N K V R S C L Stop K Y I F K N I K Stop I S
Stop R R R K E **Met** K K I W L S R K V F L Y W A E T L C Q T G A N N L F F G T G T
R L T V I P ...

J_α40(39930 to 39990)

40 N Y K I **Met** S W V C L C G S Stop T G S R G E S Stop **Met** E Y F R G F N S H L D A
Stop V L I C S L N Q T L Stop L I N **Met** H K D S Met R L K N F C K L G P N R S S E
D F L Y E L R Y N P K **Stop** I T C R K I R G Q G L S **Met** G K V H V **Met** P L L F **Met**
E S K A A S I N G N I **Met** L V Y V E T H N T V T T S G T Y K Y I F G T G T R L K V
45 L A ...

B3

J_α41(37899 to 37961)

50

QLLSL Stop YLPPTFTLEPHRIVSVHAPGCSQSRPARRSAGR
KTPDFITCHRAPSLRWQISILITHITVGSGLVSNGL *Met EEG*
SFIYTIKGPWMetTHSLCDCCVIGFQTLALIGIIGEGTWLQLQ
GVFCLGRTHCGTQIPGMHSTSAKAPRCWSHP...

5

J_α44(35064 to 35126)

10 - L G P I T H Q V Stop Q E G F I K I K P R N R K D K E F N S Q C L Q S Stop T Stop Q
L L S L N H L V S T P Stop P T E V K E G N Q Q V Met L V K Stop V S G Q S Q L P S
Stop E L I L W S L G K G N A S V R A H P G C P S G R D H G E S S E Stop G S E H
Q Met E S Q A T G F C Y E A S H S V N T G T A S K L T F G T G T R L Q V T L ...

15

Having now generally described the invention, the same will be more readily understood through reference to the following example, which is provided by way of illustration and is not intended to be limiting of the present invention.

20

EXAMPLES

MATERIALS AND METHODS

Human Cell culture

293T cell line were grown in Dulbecco's modified Eagles medium (DMEM)
25 supplemented with 10% fetal calf serum FCS (Beth Haemek, Israel), supplemented with
20mM L-glutamine, 60 μ g/ml penicillin, 100 μ g/ml streptomycin and 50mg/L Kanamycin.
Amniotic fluid cells were grown in AMF medium (Biological industries, Beit Haemek,
Israel).

30 ***GFP-TCRj2.3-C $β$ Expression Vector***

The cDNA of human TCR J2.3-C $β$ was amplified from cDNA from Amniotic fluid cells
and from cord blood mononuclear cells using the sense primer
5'CCGGAATTCCATGGGGCTCTCAGCGGTGG and antisense primer 5' CGCGGA
TCCCTAGCCTCTGGAATCCTTCTC and ligated into EcoRI and BamHI digested and
35 calf intestinal alkaline phosphatase-treated pEGFPC1 (Clontech, Palo Alto, CA). DNA
sequence analysis of the GFP-TCR J2.3- C $β$ confirmed the intended reading frame.
Proceeding from the N to C terminus, the resulting fusion protein consists of GFP a linker
sequence of 10 amino acids, and TCR J2.3-C $β$

Transfections

293T cells were plated at 70% confluence in 6 well plates and transfected with 1.6 μ g of GFP-TCR J2.3- C β construct using the calcium phosphate transfection method.

5

Western Blot Analysis and Fluorescence Analysis

For immunoblot analysis, 24 hrs after transfection 5×10^5 293T cells were lysed on ice in Tris pH 8 20mM containing 1% Triton, 140mM NaCl, 10% glycerol, 1mM EGTA, 1.5 mM MgCl₂, 1mM Sodium vanadate. Cell lysates were clarified by centrifugation at 10 15,000 g for 10 min at 4°C, and boiled after addition of SDS-sample buffer (5% glycerol, 2% SDS, 62.5 mM Tris-HCl pH 6.8, 2% 2-mercaptoethanol, 0.01% bromophenol blue).

Extracts were subjected to 12% SDS-PAGE, blotted and probed with Anti-GFP monoclonal antibody JL-8 (Clontech, Palo Alto, CA) and secondary antibody was goat anti-mouse-HRP (Sigma). Chemiluminescent signals were generated by incubation with 15 the ECL reagent. The gels were exposed to X-ray film.

(a) Cell lines and culture:

Several cell lines used herein in the examples originated in the inventors' laboratory or were obtained from other sources: mesenchymal MBA-13, MBA-15, 14F1.1, 20 NIH/3T3, AC-6, AC-11 and FBMD-1 cells; control C2C12, 1C8, MPC-11 and AB-8 cells; and MC/9 mastocytoma cells.

The cell lines were cultured by standard procedures such as in DMEM containing 10% FCS or with RPMI 1640 (GIBCO) containing 7% FCS, 2 mM l-glutamine, 5×10^{-5} M 2-mercaptoethanol and 1 mM sodium pyruvate (B). Cell lines were cultured in DMEM 25 containing 10% FCS and D-9 CM containing IL-3 and IL-4, or cultured in DMEM containing 20% FCS.

(b) Primary cell cultures:

(i) Bone marrow: Mouse bone marrow cells were obtained from femur and tibia of 30 1-2 week old female C57BL/6 mice. Bone marrow cells were removed aseptically by flushing culture medium through the marrow cavity using a 1ml syringe fitted with a 27-gauge needle. 1×10^7 cells/ml were seeded in DMEM with 20% FCS (Bio Lab, Israel) and cultured for 4-5 days at 37°C and 5% CO₂ atmosphere. The plates were washed and

covered with fresh culture medium. After 3 weeks, a monolayer was formed. The cells were passaged monthly at a split ratio of 1:10 using 0.5% trypsin (Sigma, St. Louis, MO) containing 0.02% EDTA.

(ii) Fetal fibroblast: Mouse embryo were chopped in PBS solution and treated with 5 0.5% trypsin and 0.02% EDTA at 37°C for 15 minutes. The supernatant was collected and treated again with trypsin for 30 minutes. The cell suspension obtained was then washed a few times, resuspended in DMEM-containing 10% FCS to a final concentration of 10⁶ cells/ml, and cultured for 4-5 days at 37°C and 5% CO₂ atmosphere. When a fibroblast monolayer was formed, it was trypsinized for 5 minutes, and the cells were 10 washed and resuspended as indicated before. This cell suspension (2x10⁵ cells/ml) was cultured again for 4-5 days and then collected.

(iii) Thymus and liver cells were obtained from Balb/c mice, 6-10 weeks old.

(c) Proliferation Assay:

15 Stromal cells were seeded at 1 x 10⁵ cells/ml on a 96-well round-bottom microplate (Falcon, CA) for 48 hours at 37°C in a humidified atmosphere of 10% CO₂ in air. The subconfluent cultures were supplemented with the relevant antibodies and incubated for an additional 48 hours. The cells were then pulsed with 1 mCi/well of [³H]-thymidine (Nuclear Research Center, Negev, Israel). After 24 hours, the cells were harvested, and 20 the incorporation of tritiated thymidine was determined. Briefly, the supernatants were aspirated, the cell monolayer was washed repeatedly with PBS to remove excess thymidine and extracted with 0.1N NaOH 0.2 ml/well. A volume of 0.1 ml of the cell extract was added to 3 ml scintillation liquid/vial (Quicksafe, A. Zinsser, Germany) and the radioactivity was counted in a liquid scintillation analyzer (1600TR, Packard, CT). 25 [³H]-thymidine incorporation reflecting the DNA synthesis was expressed as the stimulation index and was calculated as the ratio of the mean of the experimental samples to the mean cpm of the control sample. Untreated cells or cells treated with irrelevant antibody served as control.

30 **(d) Antibodies:**

The following monoclonal antibodies (mAbs) were used in the experiments: fluorescein isothiocyanate (FITC)-mAb anti-CD3ε (clone 145-2C11); low azide no endotoxin or FITC-conjugated hamster anti-mouse TCRβ (clone H57-597); phycoerythrin

(PE)-conjugated hamster anti-mouse TCR $\gamma\delta$) GL-3). All antibodies were purchased from PharMingen, San Diego, CA. Goat anti-human IgM (Kalestab, Denmark), FITC-conjugated goat anti-mouse (Sigma, Israel) and mouse anti-rat IgG (Jackson Immunoresearch Labs, West Grove, PA) served as control antibodies. Hybridoma supernatants of anti-TCR β (clone H57-597) and anti-CD3 ϵ (clone 145-2C11) were used for activity assays. FITC-conjugated goat anti-hamster IgG was purchased from Jackson Immunoresearch Labs. Anti-rabbit FITC Fab fragments was used as a second antibody to detect staining with rabbit polyclonal anti-peptide 1121 and anti-J β 2.6 peptide (b) antibodies.

10

(e) Flow Cytometry:

Cells were washed with PBS without Ca $^{+2}$ and Mg $^{+2}$ containing 0.02% sodium azide and incubated for 30 minutes at 4°C with FITC-conjugated anti-mouse CD3 ϵ (clone 145-2C11) or FITC-conjugated TCR β (clone H57-597) or anti-J β 2.6 peptide (b) antibodies. As second antibody for anti-J β 2.6 peptide (b) antibody, FITC-conjugated donkey anti-rabbit IgG was used (Jackson Immunoresearch Labs). For intracellular staining, cells were fixed and stained with TCR β using the Cytoperm kit (Serotec, UK). In all experiments, cells stained with isotype-matched control immunoglobulins were also prepared as negative controls for the surface and the intracellular staining. After washing with PBS, cells were analyzed for fluorescence with a FACScan (Becton Dickinson) with logarithmic intensity scales. In most cases, 5 x 10 3 cells were scored using Lysis II software (Becton Dickinson).

(f) Immunofluorescence:

Stromal cells were seeded at 10 5 cells/ml in chamber slides (Labtec slides: Nunc, USA) (400 ml/well) and incubated for 24 hours at 37°C in a humidified atmosphere of 10% CO $_2$ in air. The slides were washed in PBS (without Mg $^{+2}$ and Ca $^{+2}$) and were either unfixed or fixed in 3.7% paraformaldehyde in PBS for 20 minutes and permeabilized with 0.5% Triton X-100 in fixing solution for 2 minutes. The cells were washed with PBS for 5 minutes and blocked with normal sheep serum for 45 minutes and then stained with the relevant antibodies for 30 minutes. After incubation, the cells were washed with PBS, stained with the fluorescent second antibody for 30 minutes, washed, embedded in 50%

glycerol in PBS and cover slips were mounted and sealed. Fluorescence was examined using a Zeiss fluorescence microscope (Zeiss, Oberkochen, Germany).

(g) RNA Isolation and Northern Blotting:

5 Total RNA was extracted by Tri-Reagent (Molecular Research Center, Cincinnati, OH). For Northern blotting, poly A+ mRNA was obtained using oligo dT magnetic columns (Promega, Madison, WI). 5-30 µg mRNA were Northern blotted and probed using standard techniques with probes for the following regions: TCR C β , TCR C α and CD3 ϵ . The probes were labeled with [32 P]-dCTP by random priming (Prime-a-Gene, Promega, Madison, WI), prehybridized at 42°C in 50% deionized formamide, 2 x Denhardt's solution, 0.1% SDS, 5 x SSPE, 100 mg/ml boiled salmon sperm DNA. Hybridization was performed at the same conditions with 1 x 10 6 cpm/ml labelled probe. Filters were washed twice with 1 x SSC, 0.1% SDS at 42°C for 30 minutes and then washed twice with 0.1 x SSC, 0.1% SDS at 55°C for 30 minutes.

15

(h) PCR Analysis:

20 Total RNAs were reverse transcribed to cDNAs by incubating purified total RNA at 37°C for 60 minutes in the presence of MMLV reverse transcriptase. The primer pairs used for CD3 ϵ were as follows: sense primer, 5'-TGCCCTCTAGACAGTGACG-3' ; and antisense primer 5'-CTTCCGGTCCGGTTCGGA-3'. The TCR derived primer pairs used were as follows:

C β 5 : 1'-ATGTGACTCCACCCAAAGGTCTCCTGTTG-3';

C β 5 : 2'-AAGGCTACCCTCGTGTGCTTGGCCAGGGC-3';

C β 5 : 3'-CATCCTATCATCAGGGGTTCTGTCTGCAA-3' ;

25 C β 5 : 5'-CATCCTATCATCAGGGGTTCTGTCTGCAA-3';

C β 5 : 6'-TTCAGAGTCAAGGTGTCAACGAGGAAGG-3';

C α 1: 5'-AAGATCCTCGGTCTCAGGACAGCACC-3' ;

C α 2: 5'-ACTGTGCTGGACATGAAAGCTATGGATTCC-3'; or

Tm: 5'-GATTTAACCTGCTCATGACG-3' .

30 For PCR, thirty cycles of amplification were carried out using the following conditions for each cycle: denaturation at 94°C for 5 minutes, annealing at 58°C for 2 minutes, and extension at 72°C for 2 minutes.

(i) Rapid Amplification of 5' and 3' Ends (RACE):

5' and 3' RACE was performed for the cloning of the TCR C β chain of MBA-13 cells using the Marathon cDNA amplification kit (Clontech, Palo Alto, CA). Adaptor 5 ligated cDNA was prepared from MBA-13 mRNA according to manufacturers' directions. Hotstart-Touchdown PCR was performed as follows: 94°C for 5 minutes (x1 cycle), 94°C for 1 minute and 74°C for 3 minutes (x5 cycles), 94°C for 1 minute and 70°C for 3 minutes (x15 cycles), 94°C for 1 minutes and 68°C for 3 minutes (x10 cycles). Specific primers were used paired to the adaptor primer of the kit. The RACE products were 10 cloned into the pGEM-T plasmid (Promega) and transfected into *E. coli* JM109 cells (Promega). DNA was purified and sequenced using an automated DNA sequencer (Applied Biosystems 373A, New England Nuclear, Boston, MA).

(j) Statistics:

15 Data are presented as the mean \pm standard error of the mean. Student's t-test was performed to determine significance.

RESULTS AND DISCUSSION

20 Figure 1 shows the nucleotide sequence of a cDNA that was cloned from the stromal/mesenchymal cell line, MBA-13, that shows a J β 2.6 flanked by an intronic J (Jint-J β 2.6-C).

25 The Jint-J β 2.6-C mRNA encodes a putative protein that according to available literature (Irving, 1998) should be capable of being expressed on the cell surface. We therefore raised polyclonal rabbit antibodies by immunizing against a peptide sequence deduced from the J β 2.6 intronic sequence as follows: NH₂-LAEPRGFVCGVE-COOH, the peptide herein designated peptide (b). For this purpose, peptide (b) conjugated to KLH was injected into 2 New Zealand rabbits using Complete Freund's Adjuvant for the first boost and Incomplete Freund's Adjuvant for additional boosts. Pre-immune serum was collected before the first boost and immune sera were collected after the additional boosts. 30 Reactivity of the serum with the peptide (b) was tested by ELISA. The serum was purified on a peptide affinity column (eluted in 0.1M glycine pH 2.5 and dialyzed to PBS). The purified anti-peptide (b) antibody was also tested by ELISA.

The immunized rabbit serum was processed by isolating the specific antibodies using a column of the immunizing peptide (b). These antibodies were then tested for their ability to recognize various cell types: MBA-13 cell strains 1, 2 and 3, mouse embryonic fetal fibroblasts and thymus cells as shown in Fig. 2. Whereas thymus cells were not 5 stained (Fig. 2F), as judged by FACS analysis, two strains of the MBA-13 mesenchymal cell lines showed prominent cell surface staining by the polyclonal antibodies (Figs. 2A, 2B). On the other hand, one strain of the MBA-13 cell line was negative (Fig. 2C). A striking finding is that we found correlation between the expression of the Jint-J β 2.6-C mRNA (Fig. 3) and the reactivity of the antiserum with the stromal cells. Thus, the two 10 cell strains that expressed Jint-J β 2.6-C mRNA, also reacted with the antibody whereas one of the strains that did not show any Jint-J β 2.6-C mRNA, also did not give any signal in flow cytometric analysis using the antibodies to the intronic peptide (b) (Fig. 2C, clone 3).

The specificity of the detection of the antigen by the antiserum was further verified using competition assays with the soluble immunizing peptide (b) that reduced the ability 15 of the antiserum to stain the cells (Fig. 2D). This strongly supports the conclusion that Jint-J β 2.6-C protein is present on the surface of the MBA-13 cells. It is noteworthy that thymocytes do express Jint-J β 2.6-C on the mRNA level but are not reactive with the antibody (Figs. 2F and 3). This in fact should be expected since most thymocytes express productively TCR β and suppression of the expression of other transcripts should occur. 20 On the other hand, in mesenchymal cells that lack recombinases, no complete TCR β molecules are formed which allow the expression of Jint-J β 2.6-C.

The above findings were made using a permanent cell line (MBA-13) derived in our laboratory. We further aimed to find out whether primary mesenchymal cells also express the Jint-J β 2.6-C mRNA. As shown in Fig. 2E and Fig. 3, primary fibroblasts from 25 mouse embryo clearly express the gene on the protein and mRNA levels.

Data base survey indicated that among the seven J β s known, also J β 2.1 can theoretically encode a molecule such as Jint-J β 2.6-C. Indeed, PCR analysis using appropriate primers detected this mRNA in the MBA-13 cell line. Among the 47 possible J α s, 9 could theoretically have a composition of intronic J with an in-frame methionine 30 codon. These sequences are shown in Fig. 4 and include: J α TA31, J α TA46, J α New05, J α S58, J α New06, J α New08, J α LB2A, J α DK1 and J α TA39. Preliminary PCR analysis indicates that at least some of these versions of the α chain also exist. In addition there are

3 possible J α molecules initiated by a methionine from within the exonic coding region (not shown).

It is known from the T cell research field that TCR β cannot operate as a functional receptor unless coexpressed with pT α . We therefore examined the expression of the latter in the mesenchymal cells. Indeed pT α is expressed by the MBA-13 cell line as judged by RT-PCR. Thus, these mesenchymal cells seem to express a pT α /Jint-J β 2.6-C complex which is structurally related to a reported TCR complex containing pT α and an experimentally truncated TCR (Irving, 1998). The latter complex has been shown to be sufficient for intracellular signaling suggesting that the complex in MBA-13 is likely to be effective in signal transduction.

The study of expression of TCR was extended to a variety of stromal cell lines derived by the laboratory of the present inventors or obtained from other laboratories, as well as to primary stromal cells from the bone marrow and primary mesenchymal cells from mouse embryos. Specific stromal cell clones, but not all clones tested, expressed TCR β . Similarly, TCR α was consistently found in particular stromal cell clones (e.g., the MBA-13 stromal cell line expressed both C β and C α , whereas the MBA-15 stromal cell line did not express C β but was positive for C α (Figs. 6A-6C). Similar TCR amplified PCR products were observed in cultured primary embryo fibroblasts (Figs. 6A-6C), indicating that the expression of TCR was not a bizarre characteristic of *in vitro* passaged stromal cell lines. Rather, TCR gene expression, as judged by PCR amplification, was common to primary mesenchymal and *in vitro* passaged cells of this origin. Indeed, bone marrow mesenchymal cells, seeded *in vitro* and selected by passaging to remove contaminating hemopoietic cells, also showed clear TCR $\alpha\beta$ fragments of the expected sizes in PCR analysis. TCR gene expression was not found in B cells, mast cells or liver cells (Figs. 6A-6B).

As shown in Figs. 7A-7B, TCR $\alpha\beta$ mRNA was detected in the MBA-13 stromal cell line and also in primary fetal and bone marrow fibroblast cultures. The sizes of the TCR α transcript corresponded to that found in thymic T cells, whereas the size of the mRNA detected by the TCR β probe was about 1.1 kb as compared to 1.0 kb and 1.3 kb detected in the thymus. Of significance is that this shorter mRNA version was consistently found in different stromal cell lines, as well as in primary mesenchymal cells. A 1.0 kb mRNA species has been reported in bone marrow-derived immature precursor T

cells. The relationship between the mesenchymal 1.1 kb mRNA species and that found in early bone marrow thymocytes remains to be examined.

The above data thus demonstrate that cells of mesenchymal origin do express TCR receptor complex on the mRNA level. In addition to expression of TCR $\alpha\beta$ mRNA, expression of CD3 ϵ , which is an essential component of the functional TCR complex, was observed (Fig. 7D). Both the size of the PCR amplified product and the mRNA detected by Northern blotting deviated slightly from the sizes detected in control T-cell-derived cDNA. Flow cytometric analysis of stromal cells using an antibody to TCR $\alpha\beta$ constant region indicated that 34% of the MBA-13 cell population was stained at low intensity fluorescence (Fig. 8). These stromal cells were negative when probed with antibodies to TCR $\gamma\delta$. Importantly, no TCR $\alpha\beta$ was observed in cell lines that did not show TCR $\alpha\beta$ mRNA. These data substantiate the above described results using antibodies to the intronic sequence of J β 2.6

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, 5 without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the 10 present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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CLAIMS:

1. A cDNA molecule encoded by a T cell receptor (TCR) gene, said cDNA molecule lacking V region sequences and comprising a constant (C) domain and a joining (J) region sequences, and a 5' intronic J sequence upstream said J region sequence including an in-frame methionine codon
- 5 2. --- The cDNA molecule according to claim 1, encoded by a TCR β gene.
3. The cDNA molecule according to claim 2, wherein the joining (J) gene sequence is selected from J β 2.1 and J β 2.6.
- 10 4. The cDNA molecule according to claim 3, wherein the joining (J) gene sequence is J β 2.1 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the sequence M E N V S N P G S C I E E G E E R G R I L G S P F L as depicted in Fig. 4.
5. The cDNA molecule according to claim 3, wherein the joining (J) gene sequence is J β 2.6 and said 5' intronic J sequence including a methionine codon codes for a peptide of the sequence M G E Y L A E P R G F V C G V E P L C as depicted in Fig. 4.
- 15 6. The cDNA molecule according to claim 5, having the nucleotide sequence depicted in Fig.1.
7. The cDNA molecule of claim 2 wherein the joining J gene sequence is the intronic J β 2.3 gene sequence coding for the peptide:
- 20 M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V
8. A cDNA molecule according to claim 1, encoded by a TCR α gene.
9. The cDNA molecule according to claim 8, wherein the joining (J) gene sequence is selected from human or murine J α genes.
- 25 10. The cDNA molecule according to claim 9, wherein said 5' intronic J sequence including a methionine codon is selected from the group consisting of:
 - (i) the intronic J α TA31 gene sequence coding for the peptide:
M A W H
 - (ii) the intronic J α TA46 gene sequence coding for the peptide:
30 M E A G W E V Q H W V S D M E C L T V
 - (iii) the intronic J α TA46 gene sequence coding for the peptide:
M E C L T V
 - (iv) the intronic J α New05 gene sequence coding for the peptide:

M T V

(v) the intronic J α S58 gene sequence coding for the peptide:

M C G S E E V F V V E S A

(vi) the intronic J α New06 gene sequence coding for the peptide:

5 M A C Y Q M Y F T G R K V D E P S E L G S G L
E L S Y F H T G G S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(vii) the intronic J α New06 gene sequence coding for the peptide:

10 M Y F T G R K V D E P S E L G S G L E L S Y F H T G G
S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(viii) the intronic J α New06 gene sequence coding for the peptide:

15 M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(ix) the intronic J α New06 gene sequence coding for the peptide:

M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

20 (x) the intronic J α New08 gene sequence coding for the peptide:

M W W G L I L S A S V K F L Q R K E I L C

(xi) the intronic J α LB2A gene sequence coding for the peptide:

M V G A D L C K G G W H C V

(xii) the intronic J α DK1 gene sequence coding for the peptide:

25 M R E P V K N L Q G L V S

(xiii) the intronic J α TA39 gene sequence coding for the peptide:

M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(xiv) the intronic J α TA39 gene sequence coding for the peptide:

M E T G R E R S H F V K T S L.

30

11. The cDNA molecule according to claim 8, wherein said 5' intronic J sequence including a methionine codon is selected from the group consisting of:

(i) the intronic J α 3 gene sequence coding for the peptide:
M L L W D P S G F Q Q I S I K K V I S K T L P T

(ii) the intronic J α 6 gene sequence coding for the peptide:
M L P N T M G Q L V E G G H M K Q V L S K A V L T V

5 (iii) the intronic J α 6 gene sequence coding for the peptide:
M G Q L V E G G H M K Q V L S K A V L T V

(iv) the intronic J α 6 gene sequence coding for the peptide:
M K Q V L S K A V L T V

(v) the intronic J α 8 gene sequence coding for the peptide:
10 M S E C

(vi) the intronic J α 9 gene sequence coding for the peptide:
M A H F V A V Q I T V

(vii) the intronic J α 11 gene sequence coding for the peptide:
M G I C Y S

15 (viii) the intronic J α 13 gene sequence coding for the peptide:
M K R A G E G K S F C K G R H Y S V

(ix) the intronic J α 14 gene sequence coding for the peptide:
M L T T L I Y Y Q G N S V I F V R Q H S A

(x) the intronic J α 24 gene sequence coding for the peptide:
20 M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V

(xi) the intronic J α 31 gene sequence coding for the peptide:
M G F S K G R K C C G

(xii) the intronic J α 36 gene sequence coding for the peptide:
M K K I W L S R K V F L Y W A E T L

25 (xiii) the intronic J α 40 gene sequence coding for the peptide:
M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

(xiv) the intronic J α 40 gene sequence coding for the peptide:
M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

(xv) the intronic J α 40 gene sequence coding for the peptide:
30 M E S K A A S I N G N I M L V Y V E T H N T V

(xvi) the intronic J α 40 gene sequence coding for the peptide:
M L V Y V E T H N T V

(xvii) the intronic J α 41 gene sequence coding for the peptide:

M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E
G T W W L L Q G V F C L G R T H C

(xviii) the intronic J α 41 gene sequence coding for the peptide:

5 M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G
R T H C

(xix) the intronic J α 44 gene sequence coding for the peptide:

M E S Q A T G F C Y E A S H S V

10 12. An antisense DNA molecule of any of the cDNA molecules of claims 1-11.

13. An expression vector comprising a DNA molecule according to any one of claims 1 to 11.

14. A host cell comprising a vector according to claim 13, wherein the host is a mammalian cell

15 15. Transfected mesenchymal human cells according to claim 14.

16. A method for inducing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a cDNA molecule according to any of claims 1 to 11, in an amount effective to induce mesenchymal cell growth.

20 17. A method according to claim 16 for wound healing.

18. A method for suppressing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a DNA molecule according to claim 12, in an amount effective to suppress mesenchymal cell growth.

25 19. A method according to claim 16 for suppression of carcinomas.

20. A polypeptide encoded by a DNA molecule according to any one of claims 1 to 11.

21. A protein capable of being expressed on the cell surface or intracellularly encoded by the nucleotide sequence depicted in Fig. 1.

30 22. A protein according to claim 19, having the amino acid sequence depicted in Fig. 1.

23. A synthetic peptide deduced from an intronic J sequence of a TCR.

24. The synthetic peptide according to claim 23 selected from the group consisting of:

- (a) M E N V S N P G S C I E E G E E R G R I L G S P F L
- (b) M G E Y L A E P R G F V C G V E P L C
- 5 (c) M A W H
- (d) M E A G W E V Q H W V S D M E C L T V
- (e) M E C L T V
- (f) M T V
- (g) M C G S E E V F V V E S A
- 10 (h) M A C Y Q M Y F T G R K V D E P S E L G S G L
E L S Y F H T G G S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I
- (i) M Y F T G R K V D E P S E L G S G L
E L S Y F H T G G S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I
- 15 (j) M I S T S H G H F Q E M Q F S I W S F T V L Q I S
A P G S H L V P E T E R A E G P G V F V E H D I
- (k) M Q F S I W S F T V L Q I S
A P G S H L V P E T E R A E G P G V F V E H D I
- 20 (l) M W W G L I L S A S V K F L Q R K E I L C
- (m) M V G A D L C K G G W H C V
- (n) M R E P V K N L Q G L V S
- 25 (o) M E V Y E L R V T L M E T G R E R S H F V K T S L; and
- (p) M E T G R E R S H F V K T S L.

25. The synthetic peptide according to claim 23 selected from the group consisting of:

- 30 i) M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V;
- ii) M L L W D P S G F Q Q I S I K K V I S K T L P T;
- iii) M L P N T M G Q L V E G G H M K Q V L S K A V L T V;
- iv) M G Q L V E G G H M K Q V L S K A V L T V;
- v) M K Q V L S K A V L T V;

vi) M S E C;
vii) M A H F V A V Q I T V;
viii) M G I C Y S;
ix) M K R A G E G K S F C K G R H Y S V;
5 x) M L T T L I Y Y Q G N S V I F V R Q H S A;
xi) M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S
V;
xii) M G F S K G R K C C G;
xiii) M K K I W L S R K V F L Y W A E T L;
10 xiv) M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V;
xv) M P L L F M E S K A A S I N G N I M L V Y V E T H N T V;
xvi) M E S K A A S I N G N I M L V Y V E T H N T V;
xvii) M L V Y V E T H N T V;
xviii) M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I
15 I G E G T W W L L Q G V F C L G R T H C;
xix) M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C
L G R T H C; and
xx) M E S Q A T G F C Y E A S H S V.

20 26. An antibody raised against a peptide according to one of claims 23 -25.
27. The antibody according to claim 26 raised against a peptide in claim 24.
28. The antibody according to claim 26 raised against a peptide in claim 25.
29. Use of an antibody according to claim 26 -28 as a marker of mesenchymal
cells.
25 30. Use of a polynucleotide or peptide sequence essentially as shown in the
specification.

For the Applicants,



Cynthia Webb

Patent Attorney

-----Intron 5' to J β 2.6-----

1	M G E Y L A E P R G F V C G V E P L	18
1	TTCCCTAAATGGGAGAATAACCTCGCTGAACCCCGCGGGTTGTGTGGGGTTGAGCCTC	60
19	C S Y E Q Y F G P G T R L T V L E D L R	38
61	TGTGCTCCTATGAACAGTACTCGGTCGGCACCAGGCTCACGGTTAGAGGATCTGA	120
39	N V T P P K V S L F E P S K A E I A N K	58
121	GAAATGTGACTCCACCCAAAGGTCTCCTTGTGAGCCATCAAAAGCAGAGATTGCAAACA	180
59	Q K A T L V C L A R G F F P D H V E L S	78
181	AACAAAAGGCTACCCCTCGTGTGCCAGGGCTCTCCCTGAACACGTGGAGCTGA	240
79	W W V N G K E V H S G V S T D P Q A Y K	98
241	GCTGGTGGGTGAATGGCAAGGAGGTCCACAGTGGGTCAGCACGGACCCCTCAGGCCTACA	300
99	E S N Y S Y C L S S R L R V S A T F W H	118
301	AGGAGAGCAATTATAGCTACTGCCTGAGCAGCCCTGAGGGTCTCTGCTACCTCTGGC	360
119	N P R N H F R C Q V Q F H G L S E E D K	138
361	ACAATCCTCGAAACCACTCCGCTGCCAAGTGCAGTCCATGGGCTTCAGAGGAGGACA	420
139	W P E G S P K P V T Q N I S A E A W G R	158
421	AGTGGCCAGAGGGCTCACCAAACCTGTACACAGAACATCAGTGCAGAGGCCTGGGCC	480
159	A D C G I T S A S Y H Q G V L S A T I L	178
481	GAGCAGACTGTGGAATCACTTCAGCATCCTATCATCAGGGGTCTGTCTGCAACCATCC	540
179	Y E I L L G K A T L Y A V L V S G L V L	198
541	TCTATGAGATCCTACTGGGAAGGCCACCCATATGCTGTGCTGGTCAGTGGCCTGGTGC	600
199	M A M V K K K N S *	208
601	TGATGCCATGGTCAAGAAAAAAATTCCCTGAGACAAACTTTATGCATCCTGAGCCGTT	660
661	CTTCACCCCTGGCCATAGATTTCTGCACCTCTCTAATTCTGTTCTAAGAACCTGTC	720
721	TCTTCTTCCTCCATGGATATCCATCCTCCTCGTTGACACCTTGACTCTGAAA	773

FIGURE 1

FIGURE 2

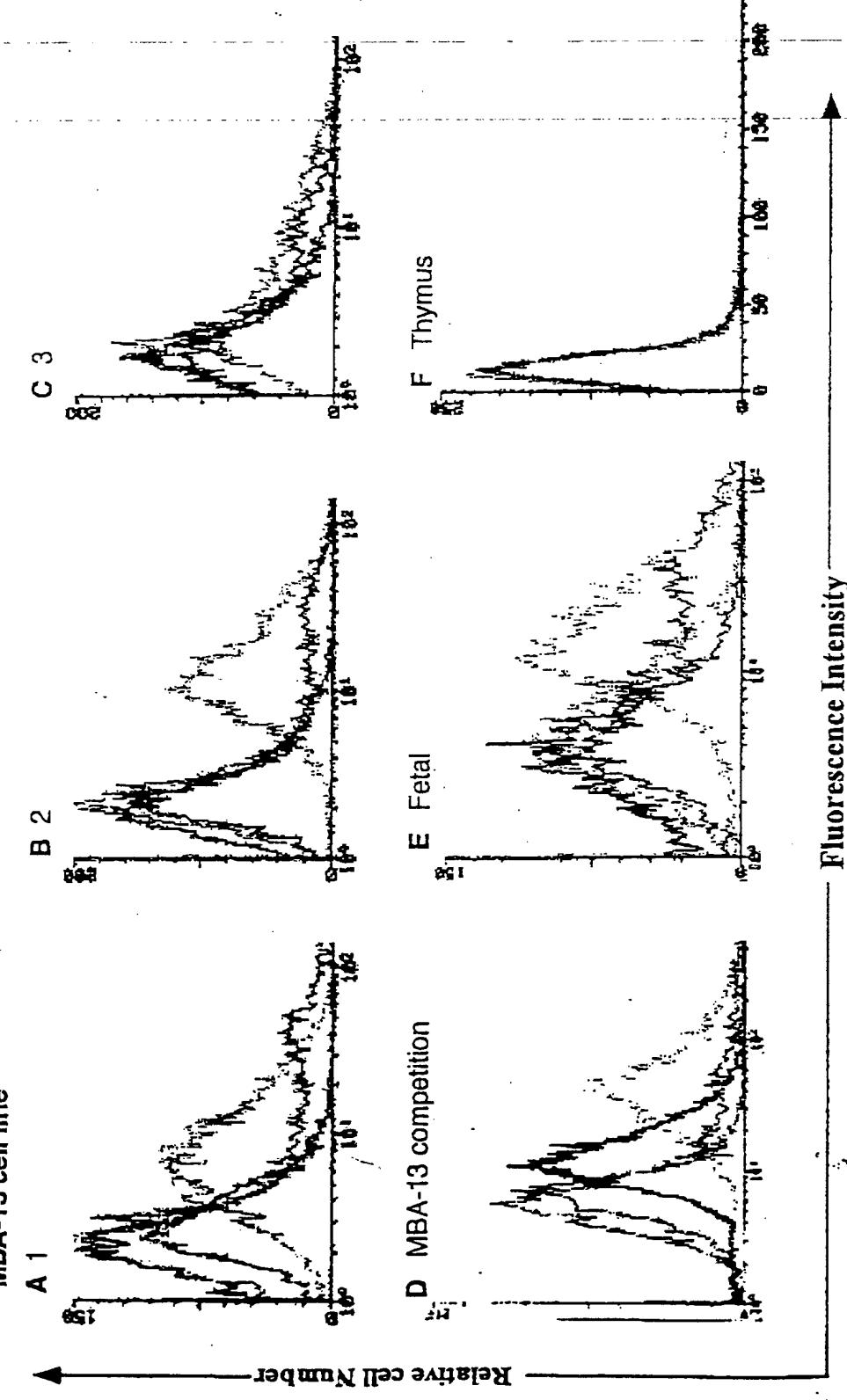
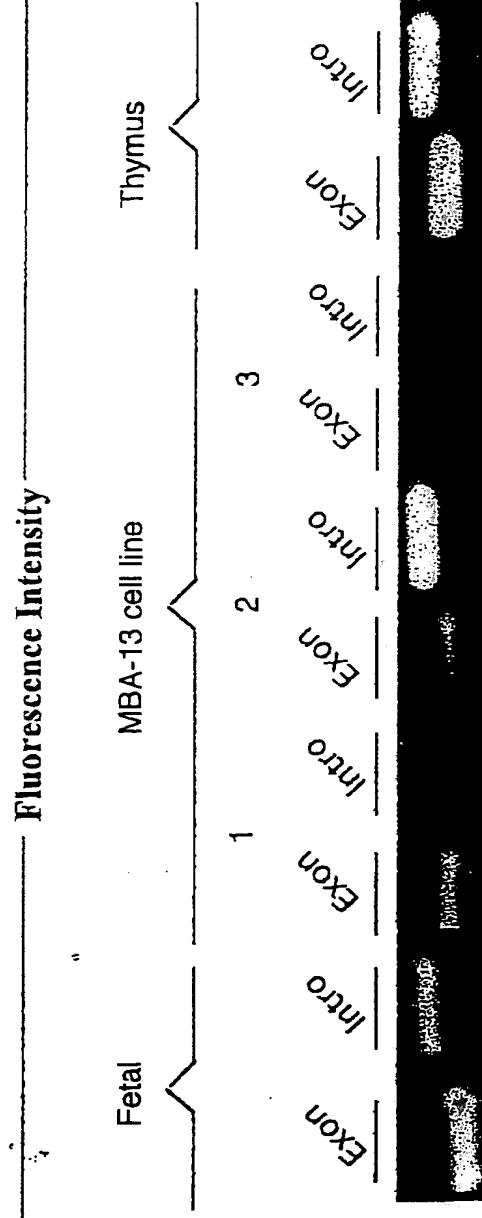


FIGURE 3



Sequences of intronic J β sequences containing Met:(Green = nomenclature; M = Met; Blue = J β exon; = continuation of C β 2).

J β 2.1 KGSREVEPPFSPYHVNHQSIRTCMGNYELIKKH
 Stop VEK Stop TLCGKEVTSPFSLEATWTPTGSLQISNSLCQ
 TLSE
 Stop MDIRSQAKSGISSL Stop DRPHARSRLPYQFWR Stop M
 ENVS NPGSCIEEGEERGRILGSPFLLCNYAEQFFGPGT
 RLTVL

J β 2.6 ELLGNCSGEFWGFWRLYPEFPSRALEREAE Stop QG
 DFP Stop MGEYLAEPRGFVCGVEPLCSYEQYFGPGTRLTV
 L.....

Sequences of intronic J α sequences containing Met:

J α TA31 VSKKKKKKSVTIL Stop NSEPAEGAINSSLGS
 LDPG Stop NVLEHCTGLLPSPKDDP Stop CQDRSSFLWGGG
 QWIFAVIVFCLAHSPRLW Stop PETSPQSTTQEQRVKG Stop
 LN
 Stop GERDIGHVRTRRNFTQKKNCHLGR Stop SVSMAEVT
 PPPCPRLVSQLRHGH Stop QKGGFLSSLKTNLAESHLPSS
 PNEPVVSVDALGSVRRVFAVAEGSRLTRRARWGRTRYRG
 WTEASPCLHSSCAA Stop SSCGF Stop TGGRGWWGRGAIPK
 AVACFGICSGLLCLPPWERTHLASRRLDVAGQEDTGVG
 GNSFRGEGERGGRTVVEGVTGGSMSRM Stop S Stop VKFK
 KLEIKNKQGRGLQKVYRAGTVDFVMAWHTVANYGNE
 K
 ITFGAGTKLTIKP.....

J α TA46 Stop VFLPGRWEPK Stop EVDRDISNPPCKPLV Stop
 LPTVDTV Stop TI Stop RTLSHIDEGSVVHT Stop EDSRDLSL
 VTVSDCMPIVVHSRVQQTKDRDIKIRWTL Stop PHLCNQ
 MIFTGSLANGCVA Stop SLTISPLLSPWLSFGSLSLT Stop NL
 K Stop
 SIY Stop IIRFLGCITHKKMTSRHININPEERGQRALSQT
 CSENLNTTPCFNQLASA YDQLRQRATDRKWSSRHHLTR
 AL Stop PHQR Stop YFRVQESFPQAGWLERGHGSALRQAME
 AGWEVQHWVSDMECLTVVTGSGGKLTGAGTRLQVN
 L.....

J α New05 Stop VKD Stop GYPKTK Stop VCGFAVLCSFGGCM
 LPPRSLCITLMGLC Stop LMKGHSKDLDEEVIIITAFFHY
 Stop LRI Stop RSA Stop R Stop FINVRLMFVLR Stop Y Stop KPNNSK
 IRLS
 SVT Stop THIHTHSHTHILTHWHNHTHTHTESQSHTHTHS
 HTSTITHLTQPHTHSLSLSLSLSLSLSLSLPRQ
 CNCIWFPSRNGCCVCLT Stop DMQSYQLVSWLGFCCY Stop
 FSVKTLPVKEAWCYQP Stop SCHYSNHIYT Stop PFYYFIS
 LKLAQLIRIQCWGNGKTSQF Stop S S Stop LHSQLLVLRG
 CSKPSQTLGTTKAARRKASTRGEDDVAFLGLPLGPSCLL
 VIVRPQMTVNSGGSNAKLTFGKGTKLSVKS.....

J α S58 WV Stop RFHVTAVALCSF Stop T SLLHLF Stop LETL
 GFRLSFLFKKQSL Stop SK Stop QDLLCLLSFHIVTKAGR

FIGURE 4

SKLGLRLLAKVEWM Stop V Stop L V Y R K E R F V L L F F Stop P Stop
 Stop Y S
 KV K A T T V A S K V L Q A W S V L Q G E T W G N W L T F H G K T G M L
 F V
 V G L L L L S S L S L K E T Stop Y N T F Stop L S G F E Stop L G I Q
 M C I T C S W Q G S R A V V L N L P N V V A P S P P K T I K L F C C Y F I A
 V T L L L Stop I G M Stop I S Y M Q L I Stop Y A T P V K G S L N P Q R R S
 A L Q D E S R C C R G R W S T V S N V R G A I E L G R N T M P T F E E K K N
 S S L G L E Q D Stop P L F L V S P L P L E K K P F I C N G L S R L M S F
 Stop M R F H V L T Stop Stop D S L G R R S L L P L Q V Stop Stop V F Stop D
 Stop V G N V N C T A K I R R A G I N S Q P L L M L S L Stop N R N Q I R M L
 S S V C V H T P P R A S Stop F D Stop C Q Stop L I Q I F R H L S E Q T S L G
 S L C L N Stop L S R Y L H N C Q I C F T L C C I D S A Stop Stop K Q M R L C
 F P R S F S P R R S S L P P S K Stop H L F T Q R E D V Q R V T Stop L I A A
 A S L H L Y D S L P W K R L K H F I R L I S Stop T D Stop Q P N Stop E E R N
 R F Stop A S F L W L Q F Q A T H L E H L V R H L R N T G A R R E V V S L C G
 L V F L S C T E N F T Q E E E S K Stop V E N Stop Q P G I H M Y T K Q S Stop
 A S A L S G S T V W F P H S P T P A P F I S N T Y I I L F S F S F E F L S A
 M P S H N P S T Y H C L S N P R M D G S G T G R V L F S G P S A E P L K K C
 R L Y P S S Stop V A T R R L G R G Q D E E K P Q E S G T A S L W Stop Y I R
 L N L S G L K C F S F H L E P M C G S E E V F V V E S A T V A D R L C K C
 A D I W I W H K S H S M S T.....

JaNew06 K C V F S C S L G L E Q Y C S L H P Q I F S R R I Q C L A L Q T L
 P V Stop P L K G S Y S F F Stop K Stop H R R I P F N V A N C G G D Stop T A Q
 G P N L C S S L L Stop G Q L C L L S H R Stop T S E S G G L F P S L A F P V D
 E V V L
 S T N F I V K D T H D R Q L L P Y F S L N K F F L C Stop Stop L Stop Q H I S
 A N E F L V I Q I N S S V T Stop T V A S Y P I I Q N S L T H H S A A A H C A
 S S N P D L H A S S N K A K R M A C Y Q M Y F T G R K V D E P S E L G S G
 L
 E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S
 F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I T V S S N T N
 K V V F G T G T R L Q V L P.....

JaNew08 Stop V M F H F L M F Stop N S L P L S Stop R C S E C R V G K L
 H M L G H G G Q H S C T G Y S T A Q P D T T S P T T G E T A P T L P P D T K
 I F L I V Y L I Stop R A K G K I K K L C P E S I L K S P R P S P P Y P H Stop S P
 A D C K
 F N V I F G S Y Stop K Stop Stop G F L C L M T P T V S L P S F I K G L L F C
 V W P L L A S W F C P H A P L C L F Q G W A G D N S F K S H F D V T D N R
 D
 K V L A K C N T A H G V F S R H T T S Q L F S S V Q K H G H S Y L M S A I Y
 S D T A K C S F K A G T R D F L W D L F L R L T M G W A F S G S S E M P S
 W
 I P A L P M E I L W S G Stop T A K P D M F L L Y R L L Q G L E I R T L R E N
 K S F G Stop M G R L L D G S I R K R N D Stop Q E E R P K K N T G Q A L G
 W
 G G V G M S R K M V T V G I Q E A G S L S Stop E G K Q G F L Stop L K V P S
 Q L S N L N Q Q G H L P F P S D F P V H V G M P L P P T M V C Stop E V G R
 G
 I D Q E Y V Stop H S Stop G P L F K H E T P E S V R G A K S L G P R R E M Q
 Q S N S S Q Q V W R S T E Q D P V L A L C L T P L A S P D H T A H P S S F S
 Stop P Q E S K V L D R E P E I P Stop P G Q V Q K G W S G A Q G W F L K T L
 W i S I Stop F L I Y N K F Stop L S Stop V I R K M F L L Stop T I P V K G K
 D N I Y R G P L L R C Q F P P W A S M W W G L I L S A S V K F L Q R K E I L
 C L P G T G S N R L T F G K G T K F S L I P.....

FIGURE 4 (CONT.)

JaLB2A Stop VIVTHPLC Stop IPPTRSIFALSSL Stop LGSL
 NVSVTPCPYLLSRYKWSKQILGFH Stop HSETDNCVLDI
 LQKEGFQSKGSHYFY Stop LTHKEAGDNWKVPGEYLGFO
 KADMAQCMHS Stop KIP Stop LTFIEYLLYACVNAPCTLSHL
 RG Stop W
 LWGRFYPTFKGKVEIVTKWLRENGGPS Stop TSSRPGCPH
 CGLSQPGSC Stop GLYRMK Stop PVVLVTTSSVLSQ Stop P
 Stop CL Stop EQGVR Stop DSLCFLDSDTLQNGECVHEQFHS
 GSMVNGQ Stop TNLKRSSLWLES Stop PFSTPLSSLPTFLS
 SWT FIS GKPLHRCLC Stop Stop RSQIKN Stop ERLSPGHTKN
 LRR Stop LFFQYLNKNSCDNGRG Stop HQRQNQKQ Stop MKR
 R
 PSFGMLLNGAVGGQAPL Stop SLESALQGLHSGSSGLR
 W
 RALWKEFLWHFRLWISCELEVLRPHDPSIEDKRVGYIC
 FFLFLLF Stop Stop PRNRPSNCQA EAYRDFTLRR Stop RT
 MISQCSKWGKKREREREREREREREREREMP Stop
 RRAR G Stop TKEVG Stop LCRGQI Stop SIEVFISSALE Stop N
 PSIM Stop VLVTEAVF Stop TGKQDQGSEGLPI Stop TLSKG
 C
 VIAF Stop Stop ERTLAVERLLLPOIICLLRC SL Stop RKSDC
 LP Stop LLGAWGKDLGKLRADRRSFSALHSQARERGWG
 MV
 GADLCKGGWHCVDRGSALGRLHFGAGTQLIVIP.....

JaDK1 Stop VCLFLWIPNLIHC Stop DKCKLFRHVSG
 VSTVPIHPDITGSKVPSHA FPVLTRKTGSSLYCWQAQ
 Stop GSRLEDASDAQQPAWDCPGRESCEMPSSLPLGIIL
 Stop LSSPT Stop
 ARPCLSVAYSI PASHTCGCANILIEASGRS Stop GSSMLL
 F Stop GKASH Stop Stop SKAG Stop LDSPPP KSLHIPGSGLQV
 QTTMLVVF Stop VLDMEPGCACLQGKHFIG Stop AISLAHL
 PVSIFF Stop ERISW Stop YSHLVHRQKDDVDVPRWHTVIW
 SQALIFPPSIFRCLSVKVISSSMSPGGRLACCPSSAVA
 WMASSCYPT Stop L Stop CIPIIH LTLYVYLLFPYS Stop MYC
 HATVMLFIVSSVSSVVP Stop TKIQRPNCLPCLKIIVLE
 KKLEFCCCLYRH Stop ELRSLAVARTGYDFCSV Stop LHTP
 Stop V Stop MREPVKNLQGLVSLCLPGRQSSDIWNRNHG
 QP.....

JaTA39 Stop VPDSW Stop L Stop RPPLSHSLYHTDDHMPYH
 SSKVELGFNEERN Stop MLLVVAVLHPMSHSMFIITLITSS
 DKRKFTRRTVTIC Stop TLVKMKVSTGAGAYCNSGYQKD
 QALARKKLNK Stop Stop VDLVKLLQIFFKNQYVSELTGEYS
 AAIALSGFSYSY GTT VVEPCKRGFHGLNSMLS LYSSNQK
 GGIPSR
 TPKREES Stop MLITSI Stop DHSRLSIFVRQHGTIYNVF
 IWGTRHH Stop RDA Stop Stop GC Stop DPLNLPQYL Stop GTVVK
 ELMVHADKHIPCMGKLSK Stop GCRTGCEQDRSCRNPRN
 N
 SSRRADPEERAQLKHIQVP Stop ICFDSCTGPALSVKRK
 CLIILHKLI Stop G Stop VNVCKNILQILKCYPHIKYGSIK
 QQKILKLGQS Stop TLLR Stop RDGVCSCGSVATGTG Stop KH
 PLSLMEVYELRVTLMETGRERSHFVKTSLTVQILGLTR
 GLELGQNSKSFQ.....

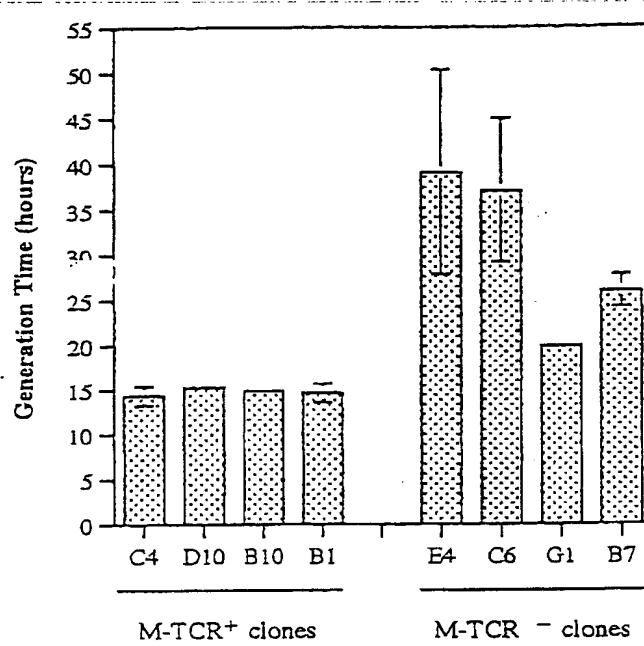
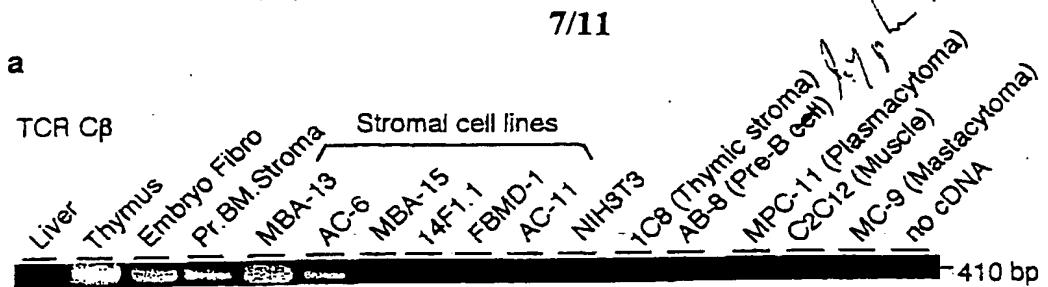


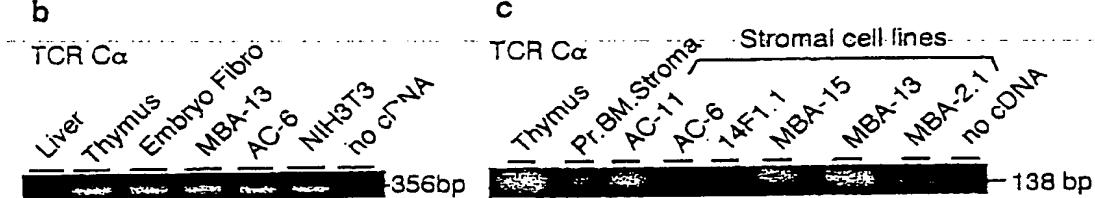
FIGURE 5

7/11

a



b



c

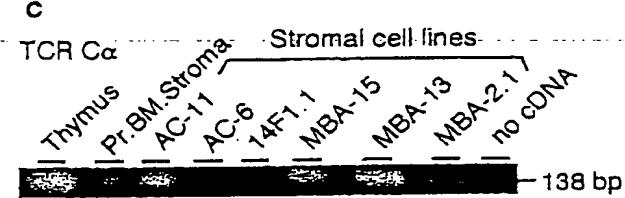
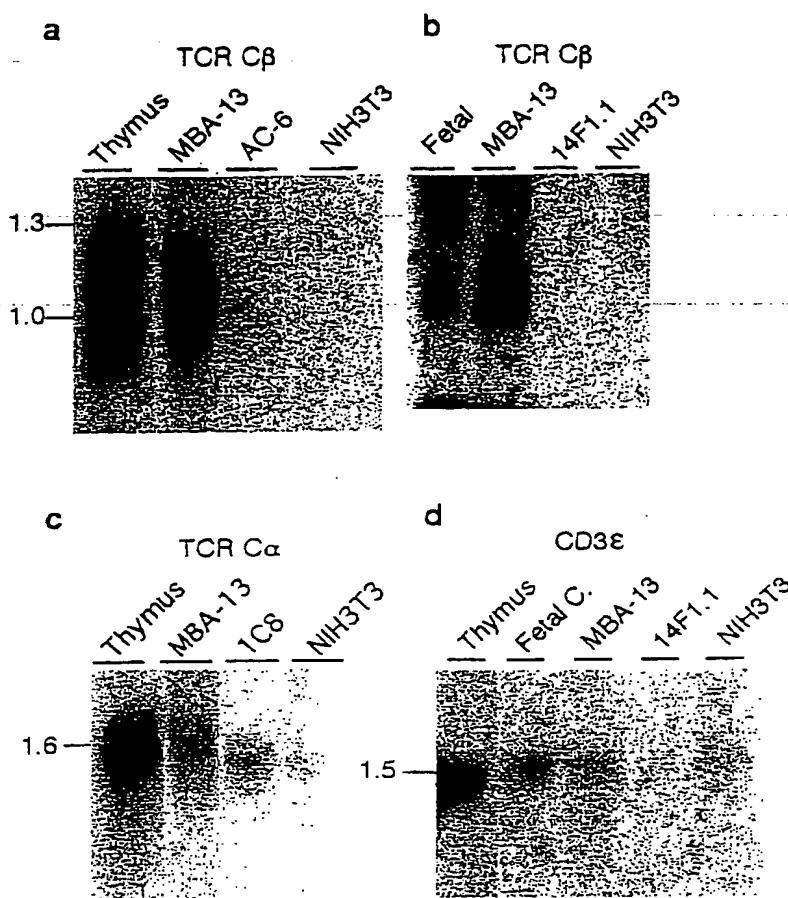


FIGURE 6

**FIGURE 7**

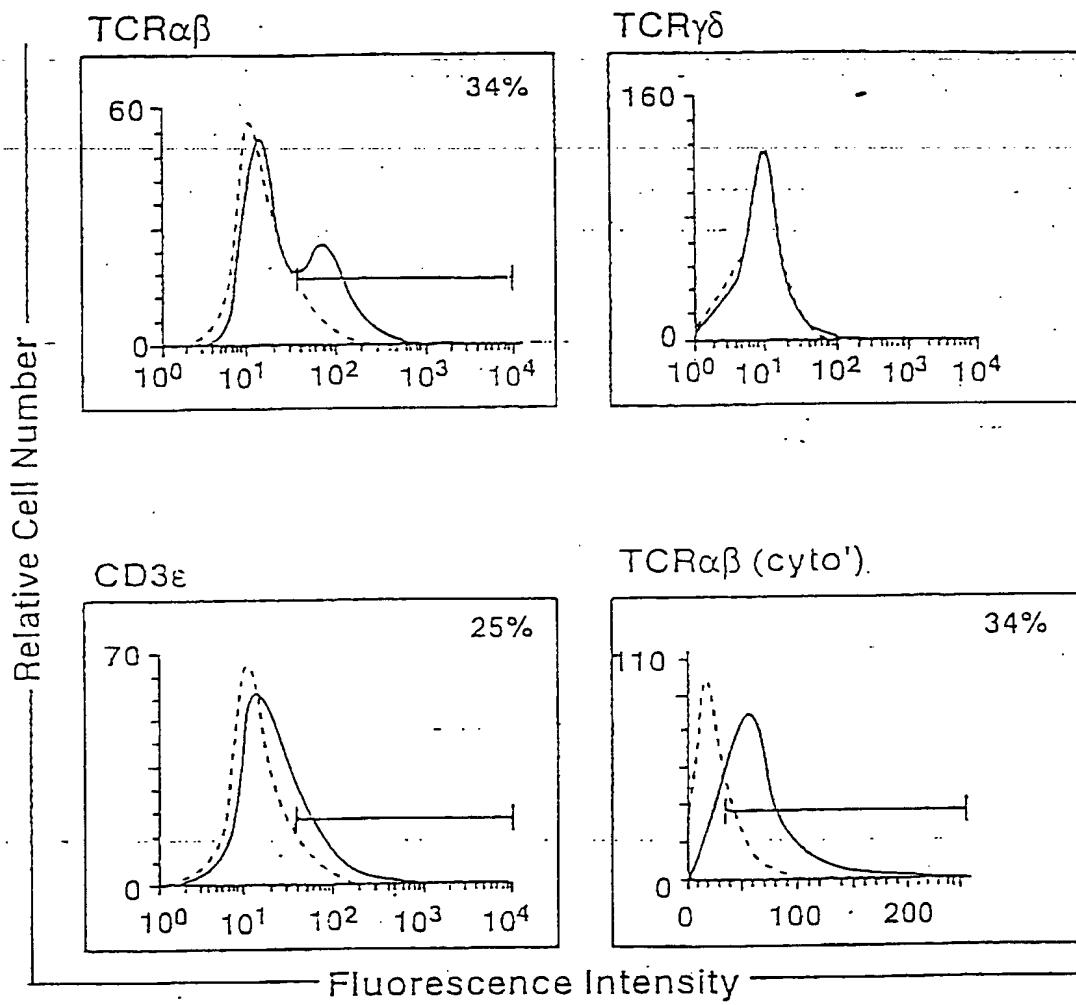


FIGURE 8

Intron 5' to J β 2.3

JB2,3

032

Intron 5' to J β 2.3

αβ2

121 accccggctgacagctgctcgaggacc:gaaaaaacg:gtttccacccggagg:cgct:gttt 130
 41 T R L T V L E D I K N V F P P E V A V F 60

181 gagccatcagaaggcagagatcccccacacccaaaaaggccacacccggcgcc 240
61 E P S E A E I S H T Q H A T L V C L A T S 90

```
501 ggggcacagcacagaccccgccggccccc:caaggcgccggccggccccc:caatgactccagatac 360
101 G V S T D P Q P C K E Q P A I N D S R Y 120
```

361 *tgccctggaggcggccctgggggtcccgccccccttcgtggcgtggaaaccccccggcaaccacttc* 420
 121 C L S S P L R V S A T F N Q N P R N H F 140

```
421 cgctgtcaaggccacggggcccgagaaatgcggatggccaccaggatggggcc 480
141 R C Q V Q F Y G L S E N D E W T Q D R A 160
```

481 aaacccgtcacccagatcgctagcgccgaggccgtggggtagagcagactgtggcttcacc 540
161 K P V T O I V S A E A W G R A D C G F T 180

541 tccgagtccttaccagcaaggggccctgtctgccaccatccctctatgagatcttgctaggg 600
 181 S E S V O O G V L S N T F V V E I L L G 200

601 aaggccacccctgtatgcgcgtgctggtcagtgcgcctcgctgtatggccatggtcaagaga 660
201 K A T L Y A V L V S A L V L M A M V K R 220

661 aaggattccagaggctag 678
221 K D S P G * 225

FIGURE 9

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